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Original Article

SPECTROPHOTOMETRIC ANALYSIS FOR THE QUANTIFICATION OF RIVAROXABAN IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

Objective

This study reveals the report on the development of two spectrophotometric methods (A and B), for the determination of rivaroxaban in bulk and Xeralto tablets.

Methods

Method A involves the formation of colored tris (1, 10-phenanthroline)-iron (II) complex, upon reaction of rivaroxaban with iron (III)-1, 10-phenanthroline mixture in acidic media. The colored complex is spectrophotometrically measured at 525 nm. Method B involves the interaction of RXN with acetaldehyde with chloranil, to give colored vinylamino substituted quinones. The colored product exhibit absorption maxima at 655 nm. Effects of experimental variables on the determination of rivaroxaban have been examined and optimized. The proposed methods are validated by following ICH guidelines.

Results

Beer's law correlating the absorbance with rivaroxaban concentration was obeyed in the range of 5-62.5 µg/ml and 10-100 µg/ml, for methods A and B, respectively. Limits of detection and quantitation values were 0. 101µg/ml and 0.306µg/ml, respectively, for method A, and their respective values for method B were 0.116µg/ml and 0.351 µg/ml. The values of accuracy, precision, selectivity, robustness and ruggedness are within the adequate limit. The proposed method was applied successfully, for the quantification of rivaroxaban in Xeralto tablets, with good accuracy and precision.

Conclusion

The proposed methods A and B are valuable, in terms of its regular application in quality control assay of rivaroxaban.

KEYWORDS: Rivaroxaban, 1, 10-Phenanthroline, Chloranil, Acetaldehyde & Spectrophotmetric Analysis

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INTRODUCTION

Rivaroxaban (RXN), chemically also known as (S)-5-Chlor-N-{2-oxo-3-[4-(3-oxomorpholin-4-yl) phenyl]-1, 3-oxazolidin-5-ylmethyl} thiophen-2-carbamide RXN is a novel, oral anticoagulant and direct Factor Xa inhibitor, that has been accepted for the management of deep vein thrombosis and pulmonary embolism [1-3]. RXN is also employed to reduce the formation of blood clots, in the legs and lungs of adult patients who had hip or knee replacement surgery [4, 5]. As a direct Factor Xa inhibitor, RXN binds directly to free and clot bound factor Xa.

After binding, it effectively blocks the amplification of the coagulation cascade, preventing the thrombus formation [6].

A variety of analytical methods have been proposed for the estimation of RXN in bulk, pharmaceutical dosage form and biological fluids. They include factor Xa specific chromogenic substrate assay [7], anti-Factor Xa chromogenic assay [8-10], prothrombin time assay [11], HPLC-MS/MS [12-14], HPTLC [15, 16], HPLC [16-26] and UPLC [27]. The above reported methods suffer from drawbacks such as time consuming, cumbersome procedure, costly and require an expertise operational personal [7-27]. Some of the methods are applicable only for the plasma samples [7-14].

Spectrophotometry is an analytical technique for the enhancement of sensitivity, simplicity, cost effectiveness and specificity in quantitative analysis of a variety of pharmaceutical compounds. In the existing literature, there are few reports regarding the use of spectrophotometry for the quantification of RXN in bulk and tablet dosage forms. Determination of RXN in the presence of its alkaline degradation products by two different approaches is proposed by Lories *et al.* [16]. The first method is the zero-crossing first-order spectrophotometry technique, in which 236 nm is chosen as λ_{max} for the determination of RXN. The second method is the first derivative ratio spectra in which the absorption spectra of RXN with different concentrations were recorded in the range of 200-400 nm and the spectra obtained were divided by a spectrum of alkaline degradates. The ratio spectra were smoothed with $\Delta\lambda$=4 intervals and their first derivatives were traced with the same $\Delta\lambda$. The concentration of RXN was determined by measuring the amplitude at λ_{max} 234 nm.

Muralikrishna & Kasad [28] and Sekaran *et al.,* [29] described UV spectrophotometric methods for the quantification of RXN. Measurement of the absorbance of methanolic solution of RXN at 248.6 nm and dimethyl sulphoxide solution of RXN at 270 nm has served as the basis for the determination of RXN, in the methods of Muralikrishna & Kasad [28] and Sekaran *et al.,* [29], respectively. The area under curve spectrophotometry technique proposed by Kasad & Muralikrishna [30], involves the calculation of integrated value of absorbance of RXN in methanol solution, with respect to the wavelength between two selected wavelengths 241 nm and 260 nm. The UV spectrophotometric methods [16, 28-30] are simple, but they suffer from lack of selectivity, as they involve measurements at shorter wavelength. Sekaran *et al.,* [29] method does not apply to tablet dosage forms.

Satyanarayana & Madhavi, have proposed five visible spectrophotometric methods (A-E), for the quantification of RXN in formulations [31]. The reaction schemes involved in the five methods are: oxidation of RXN with ferric chloride and the estimation of Fe (II) produced after chelation with 2,2'-bipyridyl (method A); Schiff's base formation of the RXN with 4-amino phenazone (method B); Charge transfer complexation of RXN with haematin formed from the reaction between haematoxyline and chloramine T, in basic media (method C); Condensation of RXN with isonicotinic hydrazide (method D); and Condensation of RXN with 1,2-napthaquinone-4-sulfonic acid sodium in alkaline media (method E). The Satyanarayana & Madhavi [31] methods suffers from one or more disadvantages like less sensitive, lack of accuracy and precision, requirement of extraction procedure and narrow range of linear response. The Satyanarayana & Madhavi [31] methods were not fully validated. The reaction optimization details, method validation parameters such as selectivity, robustness and ruggedness were not reported in the methods of Satyanaryana & Madhavi [31].

Hence, the need arises to develop certain sensitive, precise, accurate, economical and fully validated visible spectrophotometric method. In the present study, an attempt has been made to develop and validate two visible spectrophotometric methods (A & B), for the determination of RXN in bulk and tablet dosage forms. Method A is based on the oxidation of RXN with Fe^{3+} , in an acidic medium and the Fe^{2+} formed reacts with 1,10-phenanthroline, to form colored

stable tris(1,10-phenanthroline) Fe^{2+} complex. Method B is based on the reaction of N-alkylvinylamine formed from the condensation of RXN and acetaldehyde with chloranil, to give colored vinyl amino substituted quinone. The performance of the reported and proposed spectrophotometric methods is tabulated in Table 1.

Table 1: Performance of Proposed and Reported Spectrophotometric Methods for the Assay of RXN

Si. No	Reagent/Sovent	Λ Max (Nm)	Linearity (µg/Ml)	Lod & Loq (µg/Ml)	Rsd (%)	Recovery (%)	Reference
1	ACN	237.4	16-224	0.52 & 1.86	1.329	99.52	16
2	ACN	amplitude at 234	16-224	0.62 & 0.65	0.639	100.84	
3	MeOH	248.6	2-12	0.09842 & 0.2982	0.023-0.915	100.85	28
4	DMSO	270	2-20	0.212 & 0.642	0.01- 0.50.	99.82-100.50	29
5	MeOH	241 & 260	2-12	0.059 & 0.179	0.297-0.537	99.31	30
6	BPD	470	2-20	0.03 & 0.1	0.47	98.17	31
7	AP	450	3-21	0.15 & 0.5	0.88	98.58	
8	HMN	740	30-90	9 & 10	0.94	98.53	
9	INH	470	5-30	0.15 & 0.5	0.52	99.30	
10	NQS	500	15-90	1.5 & 5	1.02	99.25	
11	1,10-PTL/$FeCl_3$	525	5-62.5	0.101 & 0.306	0.112-0.450	99.78-100.80	**Proposed method A**
12	CH_3CHO /CRL	655	10-100	0.116 & 0.351	0.230-0.418	99.30-100.20	**Proposed method B**

ACN-Acetonitrile; **MeOH**-Methanol; **DMSO**-dimethyl sulphoxide; **BPD**-2, 2-Bipyridine; **AP**-4-Amino phenazone; **HMN**- Haematoxylin; **NQS**-1, 2-Naphthaquinone-4-sulphonate; 1, 10-**PTL**-1, 10-Phenanthroline; **CH_3CHO/CRL**-Acetaldehyde/Chloranil

EXPERIMENTAL

Instrumentation

- Spectrophotometric measurements were done using ELICO (Hyderabad, India) double beam model SL 159 digital spectrophotometer, with one cm matched quartz cells.
- Kemi (Ernakulam, India) KWB 220 model water baths were used to control the temperature.
- Essae-Teraoka electronic weighing balance (Goa, India) PG1000 model was used for weighing the samples.

Reagents

Method A

- **0.2% (*w/v*) 1,10-Phenanthroline (1,10-PTL):** Prepared by dissolving 0.2 gm of 1,10-PTL (Qualigens Fine Chemicals, Mumbai, India) in 100 ml of water containing 2 ml of 1M HCl (Fisher Scientific, Mumbai, India).
- **0.5% (*w/v*) Ferric chloride:** Prepared by dissolving 0.5 gm of $FeCl_3$ (Sdfine-Chem limited, Mumbai, India) in 100 ml of water.
- **0.2 M (*v/v*) Orthophosphoric:** Prepared by diluting 12.8 ml of 85% H_3PO_4 (Merck, Mumbai, India) to 100 ml with distilled water in a volumetric flask (100 ml).

Method B

- **0.5% (*w/v*) Chloranil (CLR):** Prepared by dissolving 0.5 gm of CLR (Merck, Mumbai, India) in 100 ml of 1,4 dioxane (Merck, Mumbai, India).

- **2% (*v/v*) Acetaldehyde:** Prepared by mixing 2 ml of acetaldehyde (Sdfine-Chem limited, Mumbai, India) with 98 ml of methanol (Merck, Mumbai, India).

Bulk and Tablet Dosage Forms of RXN

- Pharmaceutical grade RXN was obtained as a gifted sample from MSN laboratories, Hyderabad, India. RXN was used as received.
- Tablet dosage forms of RXN (Xeralto tablets, Bayer India Limited, Mumbai, India, labeled to contain 10 mg of RXN/tablet) were employed in the present investigation.

Stock and Working Standard Solutions of RXN

A stock standard solution containing 1 mg/ml of RXN was prepared in methanol. Working standard solution equivalent to 250µg/ml (method A) and 500µg/ml (method B) of RXN was obtained by appropriate dilution of stock solution with methanol.

General Analysis Procedure

Method A

Different volumes (0.2-2.5 ml) of RXN (250 µg/ml) were pipetted into a series of boiling tubes. The volume in every tube was brought to 2.5 ml, by adding methanol. To each tube, 1 ml of 0.5% $FeCl_3$, 1.5 ml of 0.2% 1,10-PTL and 1 ml of 0.2 M H_3PO_4 were added, mixed well, and heated on a water bath maintained at a temperature of 70°C for 20 min. The tubes were cooled at room temperature, and then the contents of the tubes were transferred to volumetric flasks (10 ml) and diluted to volume with methanol. The absorbance of the red colored solution was measured at 525 nm against a reagent blank treated similarly except without RXN.

Method B

Aliquots of (0.2–2.0 ml) standard drug solution (500µg/ml) of RXN were transferred into a series of volumetric flasks (10 ml) and the volume in each flask was brought to 2 ml by adding methanol. One ml of 2% acetaldehyde and 1 ml of 0.5 % CLR were added to each flask. The contents of each flask were mixed well and allowed to stand at room temperature (25±1^{o}C) for 30 min. The volume in each flask was diluted to the mark with methanol. The absorbance of the blue colored species was determined at 655 nm against the reagent blank prepared similarly omitting the RXN.

Construction of Calibration Curve

For both the proposed methods (A and B), the calibration curves were constructed by plotting the absorbance against the final concentration of the drug. The corresponding regression equations were derived. The concentration of the unknown samples was read from the corresponding calibration graph or computed from the corresponding regression equation.

Procedure for the Analysis of RXN in Tablet Dosage Forms

Twenty Xeralto tablets (Bayer India Limited, Mumbai, India) claimed to contain 10 mg of RXN were weighed and pulverized. An amount of powder equivalent to 100 mg of RXN was weighed into a 100 ml volumetric flask, 50 ml of methanol was added and shaken thoroughly for about 10 min. The volume of the flask was diluted up to the mark with the same solvent, mixed well and filtered using a quantitative filter paper. The filtered solution was suitably diluted with the

methanol. Convenient aliquots were subjected to analysis of the procedures described under methods A and B. The nominal content of RXN in the tablet was calculated from the corresponding calibration curve or regression equation.

RESULTS AND DISCUSSION

Method Development

Method A

Ferric salts (ferric chloride) play an important role in the colorimetric determination of organic compounds. Being an oxidant, a ferric salt is converted into ferrous salt. The ferrous salt can easily be identified by the usual reagent for divalent iron, potassium ferricyanide [32], 1, 10-PTL [33], 2, 2'-bipyridyl [34], 2, 2', 2'-Terpyridyl [34] or triazine [35]. 1, 10-PTL forms a complex of low functional value with Fe (III) which acts as a better oxidant than Fe (II). The reduction product is tris complex of Fe (II), renowned as Ferroin. Based on its complexion tendency with 1, 10-PTL and oxidizing properties, ferric salts were suggested in the estimation of pharmaceutically significant compounds [36-39].

The literature survey of the analytical applications of 1, 10-PTL indicates that this reagent has not been used for the determination of RXN. The present work is an attempt to provide a new spectrophotometric method for the determination of RXN in bulk and tablet dosage forms using 1, 10-PTL and ferric chloride as analytical reagents. The proposed method A is based on oxidation of RXN by Fe^{3+} in $FeCl_3$. The resulting Fe^{2+} complexes with an unshared pair of electrons on each of the two nitrogen atoms of 1,10-PTL to produce red colored chromogen having a maximum absorption at 525 nm, against the reagent blank. The produced chromogen was stable for 60 min. Fe^{3+} interferes to a less extent in the determination of Fe^{2+}, by method A. The reactivity of the interfering Fe^{3+} has been decreased, by complexing it with orthophosphoric acid. The probable reaction mechanism is proposed in Figure 1.

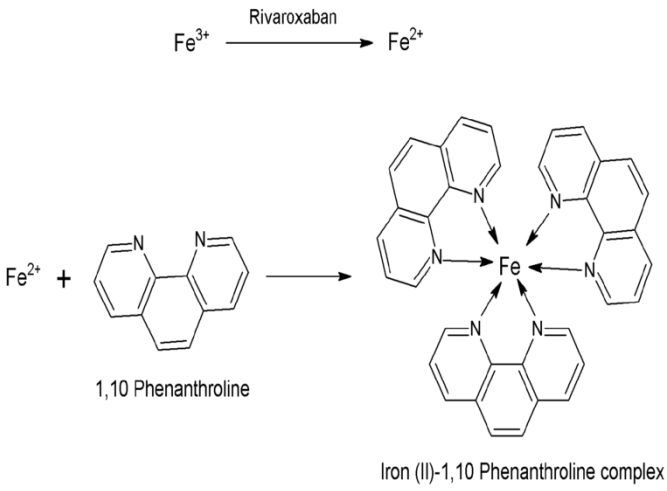

Figure 1: Reaction of Rivaroxaban with $FeCl_3$/1, 10-Phenanthroline (Method A)

Method B

Acetaldehyde interacts with primary or secondary amino group, forming N-alkylvinylamine. N-alkylvinylamine undergo condensation with halogenated Quinones (i.e., chloranil, bromanil, 2,3-dichloronaphthoquinone, 2,3-dichloro-naphthoquinone etc.,), to form colored mono alkyl amino vinylquinones, which can be measured spectrophotometrically [40]. This reaction was applied for the determination of many pharmaceutical substances [41-44]. The detailed literature survey indicated that, the color reaction (formation of monoalkylaminovinylquinones) has not yet been reported for RXN.

Therefore, in the present study this reaction has been used to develop a novel spectrophotometric method, for the quantification of RXN using chloranil as analytical reagent.

The method B is based on the formation of a colored N-vinyl chlorobenzoquinone derivative of RXN, by its secondary amino group reaction with the CLR in presence of CH_3CHO. The formed blue color showed maximum absorption at 655 nm against the reagent blank. The blue color was stable for 90 min. The probable reaction mechanism is given in Fig. 2.

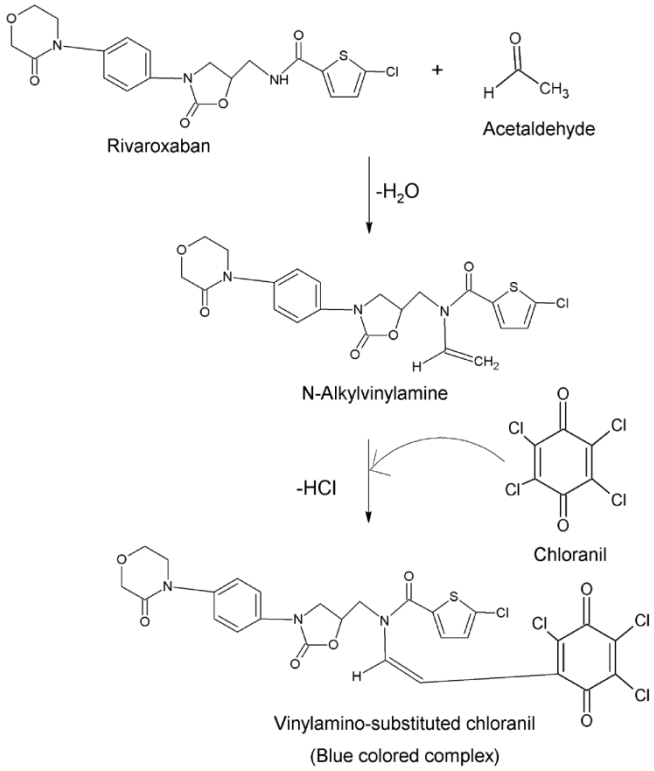

Figure 2: Condensation Reaction between Rivaroxaban and Chloranil in the Presence of Acetaldehyde (Method B)

Optimization of Experimental Variables

Method A

The experimental conditions were established by studying the effect of various parameters like concentration of 1, 10-PTL, $FeCl_3$, H_3PO_4 and temperature & heating time for the maximum color development.

Effect of Concentration of 1, 10-Phenantroline

The effect of the concentration of 1, 10-PTL was assessed by treating 25µg/ml RXN with varying volumes (0.5-3 ml) of 0.2% 1, 10-PTL. The absorbance of red colored product at 525 nm was increased with increasing the volume of 0.2% 1, 10-PTL up to 1.5 ml; above this volume, the absorbance remains constant. Therefore, 1.5 ml of 0.2% 1, 10-PTL was used in all the further measurements.

Effect of Concentration of Ferric Chloride

The influence of the concentration of $FeCl_3$ on the absorbance at 525 nm was investigated by treating 25µg/ml RXN with varying volumes (0.2-1.6 ml) of 0.5% $FeCl_3$. The absorbance was increased with increasing the volume of

NaOH and became constant at 1ml; beyond this volume, the absorbance remained constant. Therefore 1 ml of 0.5% $FeCl_3$ was suggested for the determination procedures.

Effect of Concentration of Orthophosphoric Acid

To investigate the effect of concentration of H_3PO_4 on the absorbance at 525 nm, different volumes (0.2-1.6 ml) of 0.2 M H_3PO_4 were treated with 1 ml of RXN (25µg). The results reveal that, the addition of 1 ml of 0.2 M H_3PO_4 gave the highest absorbance, after which there is no change in the absorbance of the reaction product. Therefore, 1 ml of 0.2 M H_3PO_4 was chosen as the suitable concentration.

Effect of Temperature

To study the effect of temperature for maximum color development, 1 ml of RXN (25µg) was mixed with 1.5 ml of 0.2% 1,10-PTL, 1 ml of 0.5% $FeCl_3$ and 1 ml of 0.2M H_3PO_4. The effect of temperature on the reaction was studied in the range of 30 to 90°C. Increasing the temperature up to 70°C caused an increase in the absorbance at 525 nm, whereas at higher temperatures, beyond 70°C, it decreased. Thus, 70°C was selected as being the optimum temperature.

Effect of Reaction Time

To determine the effect of reaction time for maximum color development, 1 ml of RXN (25µg) was mixed with 0.2% 1, 10-PTL (1.5 ml), 0.5% $FeCl_3$ (1 ml) and 0.2M H_3PO_4 (1 ml). The contents of the mixture were heated on a water bath at 70°C for varied time (5-30 min). The maximum intensity of color was obtained at 20 min. After 20 min, the absorbance decreased. Therefore, the optimum reaction time was fixed as 20 min throughout the experiment.

Method B

The experimental conditions were established by studying the effect of various parameters like concentrations of acetaldehyde & CLR and reaction time for the maximum and stable color development.

Effect of Concentration of Acetaldehyde

The effect of the concentration of CH_3CHO on the color development was studied by adding different volumes (0.5-3.5 ml) of 2 % CH_3CHO to 1 ml of RXN (50 µg). It was found that, the absorbance at 655 nm was reached maximum with 2 ml of 2 % CH_3CHO, and remained constant with higher volumes. Therefore, 2 ml of 2 % CH_3CHO was preferred as an optimum concentration.

Effect of Concentration of Chloranil

The effect of CLR concentration on the color development was studied by treating 1 ml of RXN (50 µg/ml) with varying volumes (0.2-1.6 ml) of 0.2% CRL. The absorbance at 655 nm was increased with increasing the volume of 0.2% CLR and became constant at 1 ml; above this volume, the absorbance remained unchanged. Thus a volume of 1 ml of 0.2% CLR was preferred for the quantification process.

Effect of Reaction Time

To study the effect of reaction time for maximum color development, 1 ml of RXN (50 µg) was mixed with 2 ml of 2 % CH_3CHO and 1 ml of 0.2% CLR. The contents of the mixture were kept at room temperature (25±1°C) for varied time (5-50 min). The absorbance at 655 nm was increased up to 30 min, and in longer times the absorbance was almost

constant. Therefore, 30 min was selected as optimum reaction time.

Method Validation

The developed methods were validated by following ICH guidelines [45].

Beer's Law and Sensitivity

Calibration graph for the quantification of RXN was prepared under the optimum experimental conditions. Method A obeys Beer's law in the concentration range of 5-62.5 µg/ml with the regression equation $A_{525} = 0.0144x + 0.0133$ (x=concentration of RXN in µg/ml). The Sandell's sensitivity and molar absorptivity of method A are 6.17×10^{-3} µg/cm^2 and 7.061×10^4 L/mol/cm of RXN, respectively.

Method B obeys Beer's law in the concentration range of 10-100 µg/ml, with the equation $A_{655} = 0.0087x + 0.0221$ (x=concentration of RXN in µg/ml). The Sandell's sensitivity and molar absorptivity of method B are 9.00×10^{-3} µg/cm^2 and 4.838×10^4 L/mol/cm of RXN, respectively.

The regression coefficient values of the standard curves for methods A and B were found to be 0.9992 and 0.9995, respectively, showing good linearity of the developed methods. The calculated limits of detection and quantitation values were 0.101µg/ml and 0.306µg/ml, respectively, for method A, and their respective values for method B were 0.116 µg/ml and 0.351 µg/ml.

Precision and Accuracy

The precision and accuracy of the proposed methods A and B were determined using intra-day and inter-day analyses of three standard concentrations of RXN (method A - 5, 37 and 62 µg/ml: method B – 10, 50 and 100 µg/ml) in replicates (n = 5). The results are shown in Table 2. The percent relative standard deviation and percent recovery values show that the precision and accuracy, respectively, is satisfactory for both the proposed methods.

Table 2: Evaluation of Precision and Accuracy of the Proposed Methods

Method	Concentration of RXN (µg/ml)		Recovery (%)	RSD (%)
	Taken	Found*		
Intra-Day Analysis				
A	5	5.03	100.60	0.401
	37	36.92	99.78	0.380
	62	62.08	100.12	0.293
B	10	10.02	100.20	0.338
	50	49.98	99.96	0.418
	100	99.93	99.93	0.268
Inter-Day Analysis				
A	5	5.04	100.80	0.450
	37	37.02	100.05	0.309
	62	62.11	100.17	0.112
B	10	9.93	99.30	0.234
	50	49.96	99.92	0.392
	100	100.08	100.08	0.230

* Average of five determinations

The accuracy of the proposed methods was further evaluated using recovery studies. Samples of tablet solution were spiked with pure RXN at three concentration levels (50,100 and 150 % of that in tablet). The total concentration of

RXN was determined by the proposed methods. The results of recovery studies were given in Table 3. The percent recovery values indicated that the recovery was good. The co-formulated substances in the tablet did not interfere in the determination of RXN by the proposed methods.

Table 3: Results of Recovery Study of the Proposed Methods

Method	Nominal Amount (mg/Tablet)	Amount of RXN Added (mg)	Found*	RSD (%)	Recovery (%)
A	10	5	14.95	0.143	99.66
	10	10	20.09	0.155	100.45
	10	15	24.95	0.595	99.80
B	10	5	14.95	0.455	99.66
	10	10	20.03	0.213	100.15
	10	15	24.96	0.152	99.88

* Average of five determinations

Selectivity

The selectivity of the proposed methods was established by observing any interference from the common tablet excipients. A placebo blank containing starch (40 mg), hydroxyl cellulose (35 mg), gum acacia (35 mg), lactose (20 mg), sodium citrate (35 mg), talc (40 mg), sodium alginate (35 mg) and magnesium stearate (35 mg) was prepared, by mixing all the excipients into a homogeneous mixture. 100 mg of the placebo blank was precisely weighed and transferred to a volumetric flask (100 ml) and its solution was prepared, as explained under section "Procedure for the analysis of RXN in tablet dosage forms". The placebo blank solution was analyzed by following the general analytical procedures. The absorbance values of placebo blank solution and the reagent blank solutions of the proposed methods were almost equal. The results obtained from analysis of placebo blank shown that common excipients used, in the tablet preparation did not obstruct the assay of RXN.

Robustness

The robustness of proposed methods was tested by changing the following experimental parameters:

Method A

- Volume of 0.2% 1,10-PTL (1.5 ± 0.2 ml)
- Volume of 0.5% $FeCl_3$ (1.0 ± 0.1 ml)
- Volume of 0.2M H_2PO_4 (1.0 ± 0.1 ml)
- Temperature (70 ± 2 ^{0}C)
- Reaction time (20 ± 2 min)

Method B

- Volume of 2% CH_3CHO (2.0 ± 0.2 ml)
- Volume of 0.5% CLR (1.0 ± 0.1 ml)
- Reaction time (30 ± 2 min)

The robustness of the methods was evaluated at two different concentration levels (method A – 5 and 62 µg/ml:

method B – 10 and 100 µg/ml). The results are shown in Table 4. The results showed no statistical differences suggesting that the proposed methods were robust.

Table 4: Evaluation of Robustness of the Proposed Methods

Method	Parameter	Concentration of RXN (µg/ml)		Recovery (%)	RSD (%)
		Taken	Found*		
A	Volume of 0.2% PTL (1.5 ± 0.2 ml)	5	5.08	101.60	0.669
		62	61.93	99.88	0.881
	Volume of 0.5% $FeCl_3$ (1.0 ± 0.1 ml)	5	5.09	101.80	0.412
		62	61.89	99.82	0.794
	Volume of 0.2M H_2PO_4 (1.0 ± 0.1 ml)	5	4.92	98.40	0.394
		62	62.11	100.17	0.974
	Temperature (70 ± 2°C)	5	4.95	99.00	0.626
		62	61.85	99.75	0.962
	Reaction time (20 ± 2 min)	5	4.98	99.60	0.532
		62	61.75	99.59	0.853
B	Volume of 2% CH_3CHO (2.0 ± 0.2 ml)	10	10.03	100.30	0.857
		100	99.92	99.92	0.611
	Volume of 0.5% CLR (1.0 ± 0.1 ml)	10	9.94	99.40	0.633
		100	100.04	100.04	0.842
	Reaction time (30 ± 2 min)	10	9.98	99.80	0.691
		100	99.90	99.90	0.445

* Average of three determinations

Ruggedness

Ruggedness of the proposed methods was determined by the analysis of RXN standard solutions (method A – 5 and 62 µg/ml: method B – 10 and 100 µg/ml)) by two different instruments and two different analysts using the same experimental conditions. The selected variables evaluated in the ruggedness testing are given in Table 5. No significant statistical difference was observed in the results, thus demonstrating that the proposed methods were rugged.

Table 5: Evaluation of Ruggedness of the Proposed Methods

Method	Parameter	Concentration of RXN (µg/ml)		Recovery (%)	RSD (%)
		Taken	Found*		
A	Analyst I	5	4.96	99.20	0.524
		62	62.05	100.08	0.621
	Analyst II	5	5.02	100.40	0.251
		62	61.94	99.90	0.458
	Instrument I	5	4.96	99.20	0.365
		62	62.08	100.13	0.458
	Instrument II	5	4.96	99.20	0.459
		62	61.97	99.95	0.624
B	Analyst I	10	9.97	99.70	0.358
		100	99.96	99.96	0.548
	Analyst II	10	10.02	100.20	0.596
		100	99.92	99.92	0.752
	Instrument I	10	100.01	1000.10	0.624
		100	100.05	100.05	0.648
	Instrument II	10	9.96	99.60	0.569
		100	100.04	100.04	0.574

* Average of three determinations

Application of the Proposed Methods to Tablets

Both the methods were applied successfully to the analysis RXN in tablets (Xeralto tablets, labeled to contain 10 mg of RXN per tablet). Satisfactory results were obtained (Table 6). The results are in good agreement with label claim. The proposed methods, therefore, are suitable for the determination of RXN in tablets with adequate accuracy and precision.

Table 6: Determination of RXN in Tablets by the Proposed Methods

Method	Labeled Claim (mg/Tablet)	Found*	RSD (%)	Recovery (%)
A	10	10.05	0.253	100.50
B	10	9.92	0.544	99.20

* Average of five determinations

CONCLUSIONS

The present study reported the successful evaluation of 1,10-phenanthroline/ferric chloride (method A) and chloranil/acetaldehyde (method B), as analytical reagents in the development of two spectrophotometric methods, for the precise and accurate determination of RXN in bulk and in its tablet dosage forms. The developed methods are fully validated, as per the ICH guidelines. The developed methods do not need expensive sophisticated apparatus. The proposed methods are sensitive, precise and accurate than the reported visible spectrophotometric methods (Table 1). The chromogens produced in methods A and B remains stable, for atleast ≥ 60 min. This gives the high throughput property, to the proposed methods. Therefore, the methods A and B are valuable for its routine application in quality control laboratories for analysis of RXN.

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